John von Neumann Institute for Computing

Single Amino Acid Contributions to Binding Affinity in Enzyme-Inhibitor Interactions: a Docking-Based Screening of BPTI-Beta Trypsin interaction

Daniele Dell'Orco, Pier G. De Benedetti, Francesca Fanelli

published in

NIC Workshop 2006,
From Computational Biophysics to Systems Biology,
Jan Meinke, Olav Zimmermann,
Sandipan Mohanty, Ulrich H.E. Hansmann (Editors)
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **34**, ISBN-10: 3-9810843-0-6,
ISBN-13: 978-3-9810843-0-6, pp. 67-72, 2006.

http://www.fz-juelich.de/nic-series/volume34

Single Amino Acid Contributions to Binding Affinity in Enzyme-Inhibitor Interactions: a Docking-Based Screening of BPTI-Beta Trypsin interaction

Daniele Dell'Orco[1], **Pier G. De Benedetti**[2], **and Francesca Fanelli**[1]

[1] Dulbecco Telethon Institute,
Department of Chemistry, University of Modena and Reggio Emilia, Italy
via Campi 183, 41100 Modena, Italy
E-mail: {*dellorco,fanelli*}*@unimo.it*

[2] Department of Chemistry, University of Modena and Reggio Emilia, Italy
via Campi 183, 41100 Modena, Italy
E-mail: deben@unimo.it

Enzyme-inhibitor interactions are crucial for normal functioning of many biological pathways. Point mutations in either the enzyme or the inhibitor molecule often lead to a modulation of the binding affinity (ΔG^o) with no major alterations in the 3D structure of the complex. The possibility to screen *in silico* the effects of point mutations on ΔG^o is of high interest, especially for protein design purposes. We have recently developed a computational protocol based on an existent rigid-body docking algorithm, which has shown a good capability to predict ΔG^o changes upon mutations in protein-protein interactions. Here, we present the results obtained for the bovine pancreatic trypsin inhibitor (BPTI) and beta-Trypsin (β-Tryp) interaction. In this system, the BPTI^{Lys15} residue was replaced by eight different amino acids, hence varying the physico-chemical nature of the interface. The X-ray structure of each variant is available, as well as the effect of each substitution on the binding energetics. We compare our approach with data arising from both *in vitro* measurements and from another structure-based empirical approach, based on changes in solvent accessible surface areas (ΔASA) upon binding. The performance and the limitations of our approach are discussed.

1 Introduction

Enzymes are the unquestioned protagonists of catalysis for virtually all the biochemical reactions occuring within cells. Inhibitors are usually proteins or other molecules specifically competing with enzyme substrates, hence exerting a regulatory mechanism of the biochemical pathway they belong to. Understanding the physico-chemical features of enzyme-inhibitor binding is fundamental, especially for designing specific molecules for therapeutical and biomedical purposes. Moreover, enzyme-inhibitor represent an interesting class of high-affinity protein-protein complexes.
Naturally occurring or artificially induced point mutations may significantly affect both the structural and the kinetic/thermodynamic features of the association between an enzyme and its protein inhibitor. Here, we report a computational study concerning the high affinity interaction between bovine pancreatic trypsin inhibitor (BPTI) and beta-Trypsin (β-Tryp), see Fig. 1. In line with *in vitro* experiments, we have used the X-ray structures of eight mutants of the residue Lys 15 in BPTI to perform a rigid-body docking simulation of BPTI/β-Tryp association. We have followed a computational protocol previously developed in our laboratory[1],which is based on the recent fast Fourier-transform (FFT) protein-protein docking algorithm ZDOCK2.3[3]. Besides the present system, we investigated other

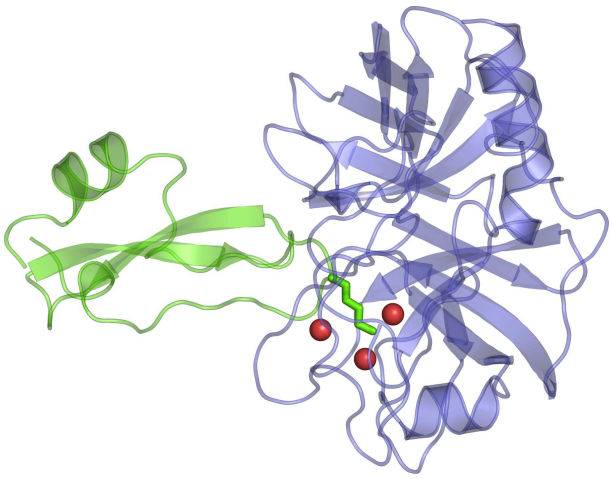

Figure 1. Three-dimesional structure of the BPTI-β-Tryp complex (Protein Data Bank (PDB) entry: 3BTK[4]. BPTI, i.e. the molecule used as a probe to sample the roto-translational space, is colored in green. β-Tryp, i.e. the molecule used as a target and hence kept fixed, is colored in slate. Structural water molecules are represented by red spheres, whereas BPTI^{Lys15}, i.e. the residue target of in silico mutagenesis, is represented in sticks.

enzyme-inhibitor complexations, namely human ribonuclease inhibitor-angiogenin interaction and barnase-barstar interaction. Our results are satisfactory for every investigated system, both in structural and in thermodynamic terms. Fairly accurate correlative predictions concerning the effects of point mutations on ΔG^o were achieved for each tested enzyme-inhibitor complex[2]. Moreover, we could gain some insights into the interpretation of kinetic data[2].

In this brief paper, we focus on the important case of a single amino acid replacement at the binding interface of an enzyme-inhibitor complex, indeed BPTI/β-Tryp. We show that accurate molecular modeling can be successfully employed in the absence of high resolution structural data, such as X-ray diffraction maps, to estimate the thermodynamic effects of specific amino acid substitutions. Finally, we compare our results with predictions arising from another broadly used empirical approach for thermodynamic analysis of protein-protein interactions starting from high resolution structures.

2 Solvent-Accessible Surface Area (ASA)-Based Empirical Approaches to Protein-Protein Interactions

The parameterization of the heat capacity, enthalpy, and entropy of protein-protein association developed by Murphy[5] and Freire[6] has been widely used in the literature to empirically calculate the free energy change from structural information. The basic assumption is that enthalpy and entropy changes, and hence ΔG^o itself, can be both linearly related to changes in the solvent accessible surface area (ΔASA) of polar and nonpolar protein atoms through empirical relationships. The well-known relationships:

$$\Delta G^o = -RTlnK_A \tag{1}$$

and:

$$\Delta G^o(T) = \Delta H^o(T) - T\Delta S^o(T) \quad (2)$$

are used to determine empirically K_A. The first assumption is that at a constant pressure, ΔH^o can be expressed in terms of the heat capacity change upon association of the protein-protein complex, by the relationship:

$$\Delta H^o(T) = \Delta H^o(T^o) + \Delta C_p(T - T^o) \quad (3)$$

where $\Delta H^o(T^o)$ is the standard reference enthalpy of association at some reference temperature T^o[5]. On the other hand, the assumption concerning the entropic term is that in ordinary conditions (e.g. no proton transfers, etc.) it may be dissected in a solvation entropy term ($\Delta S_{solv}(T)$), a conformational entropy term (ΔS_{conf}) and an overall rotational/translational entropy term (ΔS_{rt}):

$$\Delta S^o(T) = \Delta S_{sol}(T) + \Delta S_{conf} + \Delta S_{rt} \quad (4)$$

Starting form the known 3D structure of a protein-protein complex, equations 1-4 thus allow for an estimate of ΔG^o, and hence, through equation 1, for a prediction of the equilibrium constant K_A.

2.1 Structure-Thermodynamics Correlations Analysis of BPTI-β-Tryp Interaction

We used the empirical method by Murphy and Freire[5,6] to predict K_A's for BPTI-β-Tryp interaction. This was possible as the X-ray structure of eight variants of BPTI^{Lys15} are available[4]. We will refer to this method as Structure-Thermodynamics Correlation (STC) approach throughout the paper.
Results from the STC analysis are reported in Fig.2A. Although for some substitutions (i.e. Gly, Thr, Glu, Met and Phe) the affinity change follows a roughly correct trend (Fig.2A), the method apparently fails when considering the overall set of mutants. Indeed, the STC method correctly predicts that the Gly substitution has the most detrimental effect on the K_A, whereas it clearly underestimates the effect of the Phe substitution. Moreover, the overall range of K_A variation is predicted to be about one pK unit, whereas it experimentally exceeds four pK units.

3 Docking Score-Based Approaches to Protein-Protein Interaction

We recently demonstrated that rigid-body docking simulations of protein-protein association may lead to quantitative models based on tested correlative equations, which can be used to predict thermodynamic and kinetic properties[1]. The method works best with high-affinity (i.e. $\Delta G^o \leq$ -9 kcal/mol) protein-protein complexes that do not drastically change their conformation upon binding[1]. The FFT-based algorithm ZDOCK2.3[3] performs a search for optimization of intermolecular complementarity and provides a score

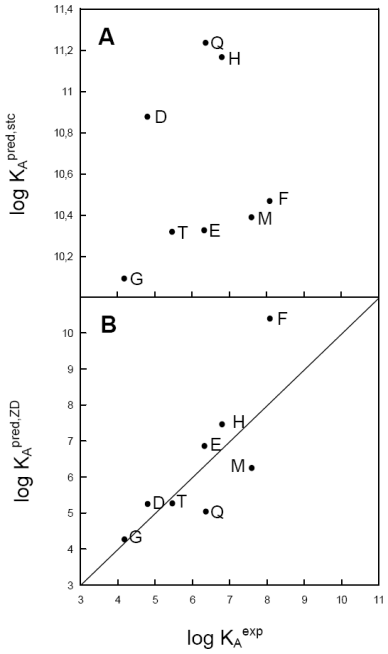

Figure 2. Comparison between the STC (**A**) and the docking-based (**B**) predictions for the equilibrium constants K_A (M^{-1}) in BPTI-β-Tryp interaction. Each individual amino acid substituting for BPTI^{Lys15} is represented by one letter code.

(ZD) for each docking solution, which can be summarized as a linear combination of three components:

$$ZD = S_{sc} + S_{el} + S_{des} \tag{5}$$

where sc, el and des indicate respectively the shape complementarity, electrostatics and desolvation terms, each implicitely including a scaling factor for the relative energy term proposed by the ZD developers[3]. In detail, we previously found a convincing correlation between the ZD score and ΔG^o for a number of protein-protein complexes that do not share structure similarity[1]. Our results, and particularly the correlative models obtained, are consistent with the interpretation of the ZD score as an empirically determined free energy function, which does not require the decomposition in enthalpic and entropic contributions. In other words, ZD can be considered as an index linearly related to ΔG^o, if we assume that this latter can be dissected as follows:

$$\Delta G^o = \Delta E_{vdw} + \Delta G_{des} + \Delta E_{el} + \Delta G_{const} \tag{6}$$

where ΔE_{vdw} represents the energy term due to van der Waals interactions, accounted for by ZD through the sc component, ΔG_{des} represents the free energy of desolvation

of atoms at the binding interface, accounted for by ZD through the des component, and ΔE_{el} represents the electrostatic interaction energy, hence accounted for by ZD through the el term. ΔG_{const} is the only term, which is not accounted for by ZD due to the rigid-body approximation. Indeed, this term includes translational, rotational and vibrational free energy changes upon binding, i.e. the conformational and the cratic entropies. The correlative context in which ZD is used, however, evidently allows such a neglection.

3.1 Docking-Based Correlative Analysis of Mutational Effects of BPTI^{Lys15} substitutions

Similarly to what we did in our previous work[1], we run docking simulations of BPTI-β-Tryp binding considering both the wild-type and mutated forms[2]. A 128x 128 x 128 point grid with a 1.2 Å spacing was used for digitalizing the interacting molecules. A rotational sampling interval of 6^o was employed and the best 4000 solutions from each run were retained and ranked according to their ZD score. Three independent sets of docking runs were performed for each complex, i.e. one starting from the X-ray coordinates and the other two randomizing the initial positions. All computations were run on a Linux cluster with 2.6 GHz Opteron CPUs.
Among the solutions generated by the algorithm, we selected as native-like structures all the docked complexes characterized by a C_a-RMSD lower than 1.0 Å from the native structure. For each docking simulation, the ZD score was averaged over all the native-like complexes resulting from the three independent runs, and employed for the correlation analysis with the thermodynamic data[2]. Figure 2B reports the predicted K_A for each BPTI^{Lys15} substitute.
The docking based-approach leads to a fair prediction of the effects of amino acid replacements in the BPTI-β-Tryp interaction, in a correlative context. Indeed, we found an acceptable correlation between ZD score and $logK_A$, with a coefficient R= 0.86 and a probability p= 0.006, when the docking was done starting from the X-ray structure of each mutant[2]. Moreover, when the mutants were modeled by introducing the substitutions in the structure of the wild type, the correlation slightly worsened (R= 0.79, p= 0.019) but the trend remained substantially the same,[2], indicative of a good potential of the method even in the absence of experimental 3D structure of each mutant.
Figure 2 reports a comparison of the results obtained in this study with the two differnt approaches presented above. A leave-one-out correlative analysis performed on ZD and $logK_A$ lead to a good prediction of the effects of BPTI^{Lys15} substitutions on the equilibrium constant (R= 0.80, slope= 1.2 for the least mean square fitting line, Fig.2B). Our approach is thus able to provide a reasonable estimate of K_A, which is consistent with the experimental values, independently of the physico-chemical nature of the substitutions.

4 Concluding Remarks

We have described a docking-based approach with an effective capability to predict the contributions of single amino acids to the binding energetics in a high-affinity enzyme-inhibitor interaction. The simplicity of the protocol, its tested capability[1,2] and the modest computational cost makes it an interesting tool also for protein design purposes. The protocol is applicable in cases of high affinity enzyme-inhibitor interations, where there is

evidence of no major conformational changes of the partners upon binding. Moreover, an assumption is made that the point mutations investigated may alter the affinity but not drastically the conformation of either the proteins involved.

Acknowledgments

Helpful technical suggestions by Emiliano Specchia and Michele Seeber are gratefully aknowledged. This work was supported by a Telethon-Italy grant (Grant S00068TELA to F.F.).

References

1. D. Dell'Orco, M. Seeber, P. G. De Benedetti, and F. Fanelli *Probing fragment complementation by rigid-body docking: in silico reconstitution of calbindin D9k*, J. Chem. Inf. Model. **45(5)**, 1429–38 (2005).
2. D. Dell'Orco, P. G. De Benedetti, and F. Fanelli *In Silico screening of Mutational effetcs on enzyme-inhibitor affinity: a docking-based approach.* Submitted for publication (2006)
3. R. Chen, L. Li, , and Z. Weng *ZDOCK: an initial-stage protein-docking algorithm*, Proteins **52(1)**, 80–7 (2003).
4. R. Helland, J. Otlewski, O. Sundheim, M. Dadlez, and A. O. Smalas *The crystal structures of the complexes between bovine beta-trypsin and ten P1 variants of BPTI*, J. Mol. Biol. **287(5)**, 923–42 (1999).
5. B. M. Baker, and K .P Murphy *Dissecting the energetics of a protein-protein interaction: the binding of ovomucoid third domain to elastase.*, J. Mol. Biol. **268(2)**, 557–69 (1997).
6. I. Luque, and E . Freire *Structure-based prediction of binding affinities and molecular design of peptide ligands.*, Methods Enzymol. **295**, 100–27 (1998).
7. V. J. Hilser, J. Gomez, and E . Freire *The enthalpy change in protein folding and binding: refinement of parameters for structure-based calculations.*, Proteins **26(2)**, 123–33 (1996).